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The importance of stimulus variability when studying face processing using Fast Periodic Visual Stimulation: A novel 'Mixed-Emotions' paradigm

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Abstract

Fast Periodic Visual Stimulation (FPVS) with oddball stimuli has been used to investigate discrimination of facial identity and emotion, with studies concluding that oddball responses indicate discrimination of faces at the conceptual level (i.e. discrimination of identity and emotion), rather than low-level perceptual (visual, image-based) discrimination. However, because previous studies have utilised identical images as base stimuli, physical differences between base and oddball stimuli, rather than recognition of identity or emotion, may have been responsible for oddball responses. This study tested two new FPVS paradigms designed to distinguish recognition of expressions of emotion from detection of visual change from the base stream. In both paradigms, the oddball emotional expression was different from that of the base stream images. However, in the 'fixed-emotion' paradigm, stimulus image varied at every presentation but the emotion in the base stream remained constant, and in the 'mixed-emotions' paradigm, both stimulus image and emotion varied at every presentation, with only the oddball emotion (disgust) remaining constant. In the fixed-emotion paradigm, typical inversion effects were observed at occipital sites. In the mixed-emotions paradigm, however, inversion effects in a central cluster (indicative of higher level emotion processing) were present in typical participants, but not those with alexithymia (who are impaired at emotion recognition), suggesting that only the mixed-emotions paradigm reflects emotion recognition rather than detection of a lower-level visual change from baseline. These results have significant methodological implications for future FPVS studies (of both facial emotion and identity), suggesting that it is crucial to vary base stimuli sufficiently, such that simple physical differences between base and oddball stimuli cannot give rise to neural oddball responses.

Keywords: Fast Periodic Visual Stimulation; Facial expressions; Alexithymia; Implicit

1. Introduction

Recognition of others' facial emotion has been extensively studied across typical, clinical, and developmental populations, due to its clear influence on social abilities. Recognising another's facial expression allows one to experience an empathic response, and behave towards the individual in an appropriate way (e.g. Besel & Yuille, 2010; Coll et al., 2017; Gery, Miljkovitch, Berthoz, & Soussignan, 2009). In the laboratory, as in everyday life, emotional expressions can be processed in an explicit, goal-directed manner, or spontaneously, in an 'implicit' manner which need not result in explicit emotion recognition (e.g. Mathersul et al., 2009; Xiao, Li, Li, & Wang, 2016). As yet, the majority of previous studies of facial emotion recognition have assessed explicit categorisation of facial expressions. Most commonly, studies have used verbal labeling tasks, but even studies that have attempted to remove language demands, for example by using matching and discrimination tasks, require explicit discrimination of facial expressions (e.g. Cook, Brewer, Shah, & Bird, 2013; Croker & McDonald, 2005; Hosie, Gray, Russell, Scott, & Hunter, 1998; Lane et al., 1996; Narumoto et al., 2000; Prkachin, Casey, & Prkachin, 2009; Stone, Nisenson, Eliassen, & Gazzaniga, 1996). Similarly, neuroimaging measures of emotion recognition without language demands often include an explicit emotion processing task, or are aided by the explicit processing of emotions (e.g. Delle-Vigne, Kornreich, Verbanck, & Campanella, 2014; Spunt & Adolphs, 2017; Vermeulen, Luminet, de Sousa, & Campanella, 2008).

Recently, Fast Periodic Visual Stimulation (FPVS) with EEG frequency tagging has been utilised to investigate implicit discrimination of facial stimuli (Dzhelyova, Jacques, & Rossion, 2017; Leleu et al., 2018). These paradigms include brief presentations of periodic stimuli, and do not require participants to engage in an explicit behavioural task using the facial images (participants are typically required to respond to changes in colour of a fixation point and do not report awareness of the periodic nature of changes in facial identity or emotion), so are thought to assess facial discrimination at an implicit level. The FPVS technique involves presenting stimulus images at a high periodic frequency rate, such that a periodic response with a high signal-to-noise ratio (SNR) is observed in the EEG signal at this specific frequency (the steady-state visual evoked potential; see Norcia, Appelbaum, Ales, Cottereau, & Rossion, 2015 for a review) and its harmonics. Importantly, when deviant oddball stimuli are presented at a specific lower frequency within a stream of identical 'base' stimuli, a response is observed at the oddball frequency if the oddball is discriminated from the base stimulus (e.g. Dzhelyova & Rossion, 2014; Liu-Shuang, Norcia, & Rossion, 2014). This technique, therefore, provides an easily quantifiable objective measure of face discrimination ability.

FPVS was first utilised to demonstrate that humans are able to discriminate implicitly facial identities. When one identity is presented in the base stream, for example at a frequency of 5.88Hz, and a different facial identity is presented as an oddball (every fifth stimulus cycle, at 1.18Hz), significant increases in SNR have been observed at the oddball frequency (1.18Hz) and its harmonics (e.g. Dzhelyova & Rossion, 2014; Liu-Shuang et al., 2014). The amplitude of oddball responses has been found to correlate moderately with performance on the commonly used Cambridge Face Memory Task assessing facial identity recognition (Xu, Liu-Shuang, Rossion, & Tanaka, 2017), and typical oddball responses were not observed to deviant facial identities in an individual with prosopagnosia ('face blindness'; Liu-Shuang, Torfs, & Rossion, 2016), suggesting that neural responses on this implicit task may contribute to more explicit face processing.

More recently, the FPVS technique has been extended to include studies of facial emotion processing. (Dzhelyova et al., 2017) examined oddball responses to happy, fearful, and disgusted facial expression oddballs among neutral base face stimuli, and found that all three emotional expressions produced oddball responses, observed over occipito-temporal regions, particularly in the right hemisphere. This finding has been extended to additional emotions (anger and sadness) and replicated using less extreme facial expressions; when extreme emotional expressions were morphed with a neutral expression, stimuli containing 40% or more of the extreme stimulus produced a neural oddball response, which increased with increasing emotional content (Leleu et al., 2018).

Importantly for the current study, facial images can be processed at a number of different levels. At the lowest perceptual level are the image properties themselves (e.g. contrast, luminance), and at subsequent levels, it is thought that recognisers construct successively more abstract, conceptual representations of facial identity and emotion. For example, under the Bruce and Young (1986) model, the recogniser constructs a viewpoint-dependent representation of the face before subsequently constructing a viewpoint-independent representation, contributing towards full recognition of the face as a particular identity. Within the emotional domain, evidence from computational models suggests that the facial expressions can be discriminated at a number of levels; 1) at a 'low-level' visual stage reflecting the visual properties of the images, 2) at a higher visual level (face-specific visual) in which the pattern of facial features are encoded as an expression, without the requirement to label this expression as indicative of a particular emotion, and 3) at the highest 'emotional-semantic' level based on recognition of the expressions as belonging to different semantic categories (Calvo & Nummenmaa, 2016; Dailey et al., 2010; Dailey, Cottrell, Padgett, & Adolphs, 2002).

FPVS neural responses to oddball facial expressions have previously been interpreted as indicative of 'high-level' detection of changes in facial emotion (Dzhelyova et al., 2017), but this level of processing has not been clearly specified thus far. The inference of high-level processing (suggestive of, at least, the intermediate processing level referred to here as 'face-specific visual') has been made because the increase in signal at the oddball frequency is substantially lower when faces are inverted or contrast is reversed (both thought to disrupt typical holistic processing of faces, and to give rise to low-level visual, feature-based processing; Dzhelyova et al., 2017; Rossion, 2008; Valentine, 1988). Further features of the design of facial FPVS studies also support the conclusion that oddball responses are not simply a product of low-level visual differences. For example, within the emotional domain, increased oddball responses to increased expression intensity remained once statistically controlling for physical dissimilarity of the image to the base neutral image (although it should be noted that the size of this effect was greatly reduced, indicating some contribution of low-level visual processing; Leleu et al., 2018). Furthermore, stimulus size (and therefore stimulation of neural populations in low-level visual regions) varied at every cycle, suggesting frequency-based responses are not a product of early visual processing. However, despite these attempts to rule out a lower-level visual encoding explanation of the oddball response, it is still possible that low-level visual detection of change from the base stream, rather than face-specific visual or emotional-semantic discrimination of facial expressions, gives rise to the neural oddball response for two reasons. First all published studies have used a single image for all base stimuli, meaning that a simple detection of differences in low-level visual image properties (e.g. a change in contrast due to the teeth being shown in the oddball, but not the base, stimulus), even when images are presented at different sizes, could give rise to an oddball neural response without the emotional content of the oddball images necessarily having been discriminated from that of the base image. Second, paradigms typically use one emotion (neutral) in the base stimulus stream and a single different emotion as the oddball. Again, detecting low-level physical differences between two emotional expressions (as expressions of the same emotion are far more physically similar than expressions of two different emotions; Gao, Maurer, & Nishimura, 2010) may be sufficient to produce a neural oddball response. As such, although oddball responses may be a product of face-specific visual or emotional-semantic discrimination of facial expressions, they may instead reflect a change in the low-level visual characteristics of oddball images from the base stream. Therefore, when FPVS paradigms utilise only two images, or even only two emotions with variable images, it cannot necessarily be concluded that oddball responses index implicit discrimination of facial emotion.

The first objective of this study was therefore to assess oddball responses to emotional expressions in an FPVS paradigm while more effectively ruling out low-level visual differences as a source of the neural oddball response. This was achieved by employing a novel ‘fixed-emotion’ FPVS paradigm in which different facial identities expressing a specific emotion are shown in the base stream, while oddball stimuli consist of different facial identities displaying a second emotion. The emotion in the base stream is therefore ‘fixed’, but the visual properties of the individual base stimuli differ from each other as they are expressed by multiple identities. The fixed-emotion paradigm is similar to existing FPVS paradigms, but includes additional variation across base images (for example, if base images are of a surprised expression, then base stimuli will be of multiple identities displaying a surprised expression) making lower-level visual explanations for neural oddball responses less likely as these lower-level visual changes are also a feature of the base stream. Note that previous paradigms have also only utilised neutral faces in the base stream, while the fixed-emotion paradigm used either neutral, surprised, or angry expressions in the base stream to increase the generalizability of the results.

The second objective of the current study was to distinguish between 1) detection of a change from the base stream and 2) recognition of a particular expression or emotion. Even when adequately accounting for low-level differences, the extent to which the neural response indexes recognition of the oddball expression/emotion, in contrast to detection of a change from the base stream, is unclear. To illustrate, consider a standard FPVS paradigm consisting of a train of base neutral stimuli with sad emotional expression oddball stimuli. Any neural oddball response may indicate *detection of a change*, either at the face-specific visual level (i.e. these stimuli were from the set of facial expressions characterised by a relaxed mouth but the stimulus changed such that the expression was no longer of this set) or at the emotional-semantic level of emotion recognition (i.e. the emotion changed from neutral). Conversely, oddball responses could be due to *recognition,* either at the face-specific visual level (i.e. these stimuli were from the set of facial expressions characterised by a relaxed mouth but the stimulus changed to one drawn from the set characterised by a downturned mouth), or at the emotional-semantic level (i.e. the expression changed from neutral to sad). To address this issue, a ‘mixed-emotions’ FPVS paradigm was created in which different images of multiple emotions are shown in the base stream, all differing from a single oddball emotion, displayed across multiple oddball images. In the mixed-emotions paradigm, the visual properties of the base stream are highly variable (base stimuli differ in emotional semantic content, as well as in identity and low-level visual characteristics) meaning that the only explanation for a signal to be observed at the oddball frequency is that oddball images have been *recognised* either as members of the same expression set (face-specific-visual), or the same specific emotion (emotional-semantic). This is in contrast to the fixed-emotion paradigm, in which base stimuli are more visually similar to each other than to oddball stimuli. The mixed-emotions paradigm therefore indexes recognition of the oddball (either at the face-specific visual or emotional-semantic level), while the fixed-emotion paradigm removes the contribution of low-level visual processing, but may still index detection of *change* from the base stream at the face-specific visual or emotional semantic level, rather than the *recognition* of expression/emotion.

To test the hypothesised differences between the fixed-emotion and mixed-emotions paradigms, a group of individuals with alexithymia completed both paradigms. Alexithymia is characterised by difficulties identifying and describing one's own emotions (Nemiah, Freyberger, & Sifneos, 1976), and has unsurprisingly been reliably associated with difficulties recognising others' facial expressions of emotion (see Grynberg et al., 2012 for a review). Although recognition of others' emotions is impaired in these individuals, alexithymic individuals are as capable as those without alexithymia at forming accurate percepts of emotional facial expressions (Cook et al., 2013). They should, therefore, perform basic detection of change from the base stream in an FPVS paradigm typically, but not recognition of an oddball category (either at the face-specific visual level or the emotional-semantic level). As it is likely that oddball responses in the fixed-emotion paradigm can occur via detection of change, but oddball responses in the mixed-emotions paradigm are only driven by recognition of either the expression category (face-specific visual level) or emotion category (emotional-semantic level), it was hypothesised that individuals with alexithymia, who are able to detect changes in facial expression, but have difficulty recognising them, would only show different neural oddball responses to non-alexithymic individuals in the mixed-emotions, and not the fixed-emotions, paradigm.

2. Methods

2.1 Participants

Forty-two right-handed participants with English as their first language and normal or corrected-to-normal vision took part in this study. This sample size was selected based on an *a priori* power analysis to provide 80% power to detect a large difference between the groups (Cohen's d = 0.9) using a two-tailed t-test and a significance threshold of $\alpha = 0.05$. Exclusion criteria were being under 18 years of age and not having English as a first language. Five participants were excluded and replaced before data analysis due to reporting a posteriori not meeting one of the recruitment criteria (n =2), technical difficulties (n=2), or extremely poor performance on the explicit facial discrimination task (n=1). The twenty participants in the Alexithymia group were selected on the basis that their score on the Toronto Alexithymia Scale (TAS-20; Bagby, Parker, & Taylor, 1994) met the cutoff of 61 for high alexithymia. The Typical comparison group consisted of twenty-two age-, gender- and IQ-matched participants scoring below the cutoff on the TAS-20. The demographic characteristics and scores on the TAS-20 of each group are shown in Table 1. All procedures in this study were approved by King's College London Psychiatry, Nursing and Midwifery Research Ethics Subcommittee, and participants received an honorarium for their participation.

Table 1. Demographic characteristics and Alexithymia scores for each group. For quantitative variables, mean and standard deviation are shown.

	Typical	**Alexithymic**
Gender (Males:Females)	13:9	11:9
Age (years)	30.14 (10.90)	29.35 (12.01)
Estimated IQ	110.55 (11.19)	108.40 (10.25)
TAS-20 total score	39.68 (7.86)	72.55 (5.11)

TAS-20: Toronto Alexithymia Scale, 20 items

2.2 Materials and Procedure

2.2.1 Self-report questionnaires and IQ estimation. Alexithymia was measured using the TAS-20 questionnaire (Bagby et al., 1994) which comprises twenty questions assessing self-reported ability to identify and describe one's feelings, as well as one's tendency to focus attention externally. The cutoff used in the current study to create the high and low alexithymia groups is based on the original authors' suggestion that a score of 61 or above indicates a clinically significant level of alexithymia (Bagby et al., 1994). To ensure that the groups were matched on general cognitive abilities, IQ was estimated using the Vocabulary and Matrix Reasoning subtests of the Wechsler Abbreviated Scale of Intelligence (WASI-II; Wechsler, 2011).

2.2.2 EEG recordings. EEG activity was acquired from a 59 channels (extended 10-10 montage) DC-coupled recording system (Brain Products, Gilching, Germany). Three additional EOG electrodes were placed below the left eye and at one cm from the outer canthi and two ECG electrodes were placed on the centre of the right and left clavicles. The sampling rate was 500 Hz, with reference at FCz and ground at AFz. Impedances were maintained below 10kΩ throughout the recording.

2.2.3 Visual stimuli. All facial expression stimuli used in this study were taken from the Radboud Faces Database (Langner et al., 2010). Stimuli consisted of static facial expressions from 20 different individuals (10 males) expressing anger, disgust, surprise, fear or a neutral emotion. The original stimuli were converted into grayscale, adjusted for differences in luminosity and cropped around the natural line of the face to remove external features (see Figure 1). The stimuli were presented over a dark grey background on a 17 inch LCD monitor with a measured refresh rate of 57.7 Hz.

2.2.4 Implicit facial expression discrimination task (FPVS). In order to measure participants' ability to implicitly discriminate facial expressions, we used a fast periodic visual stimulation task with oddball stimuli (Figure 1A). During this task, participants were presented with facial expressions at a rate of 5.77 Hz (base frequency). A base facial expression was presented 80% of the time and every fifth stimulus (1.154 Hz; oddball frequency) depicted an oddball facial expression.

As in previous studies (e.g. Dzhelyova et al., 2017), the contrast of the stimulus was sinusoidally modulated in order to create a smoother onset and offset of the stimulus and the size of the stimulus was randomly varied within a range of 90-110% of its original size at each presentation. Furthermore, the identity of the facial expression was randomly selected among the 20 available identities to reduce low-level visual similarity between the faces. Stimuli were presented in trials of 83.2 s comprising 96 cycles of five faces, whereby a total of 480 faces were each presented for 173.3 ms. In order to avoid an abrupt start/end of the visual stimulation, the maximum contrast of the stimuli in the five cycles at the start/end of the task was gradually increased or decreased.

Four oddball/base expression pairs were selected for the purpose of this study. The first three represent fixed-emotion paradigms (Fear/Neutral, Fear/Surprise, Disgust/Anger) and the fourth was a mixed-emotions paradigm (Disgust/Random). In the Disgust/Random pair, each base stimulus was randomly selected among Neutral, Fear, Anger, Happy, Sad and Surprise expressions, while the oddball expression was always Disgust (randomly selected at each presentation from the twenty facial identities). Each of the pairs was presented once with the faces upright and once with the faces inverted, yielding a total of eight trials. The presentation order of the trials was fully randomised.

A concurrent task was used to ensure attention to the stimuli. Participants were instructed to fixate a central cross located between the eyes on the face stimuli and to click the mouse each time they noticed that the fixation cross changed from black to red. The cross was red for 333 ms 10 times in each trial and each change in colour was separated by at least 333 ms.

2.2.5 Explicit facial expression discrimination task. Participants' ability to purposefully discriminate facial expressions based on visual differences was assessed using an explicit facial expression discrimination task (Figure 1B). Each trial of this behavioural task showed nine facial expressions presented at the same rate and with the same parameters as in the implicit categorisation task. In 50% of the trials, the fifth expression displayed an oddball emotion. Participants were explicitly told the oddball emotion to look for and the base emotion to discriminate from before each trial (e.g., "Look for Anger in Disgust"). After each presentation, participants were asked to indicate as quickly as possible if the target emotion was present or not using the 1 and 2 keys on the numeric keypad of a standard keyboard. For the explicit categorisation task, participants were asked to detect Fear in streams of Surprise, Surprise in streams of Fear, Anger in streams of Disgust and Disgust in streams of Anger. Forty trials were performed for each of the four pairs for a total of 160 trials. Trials were blocked according to the base/oddball pair and the presentation order of the trials was fully randomized.

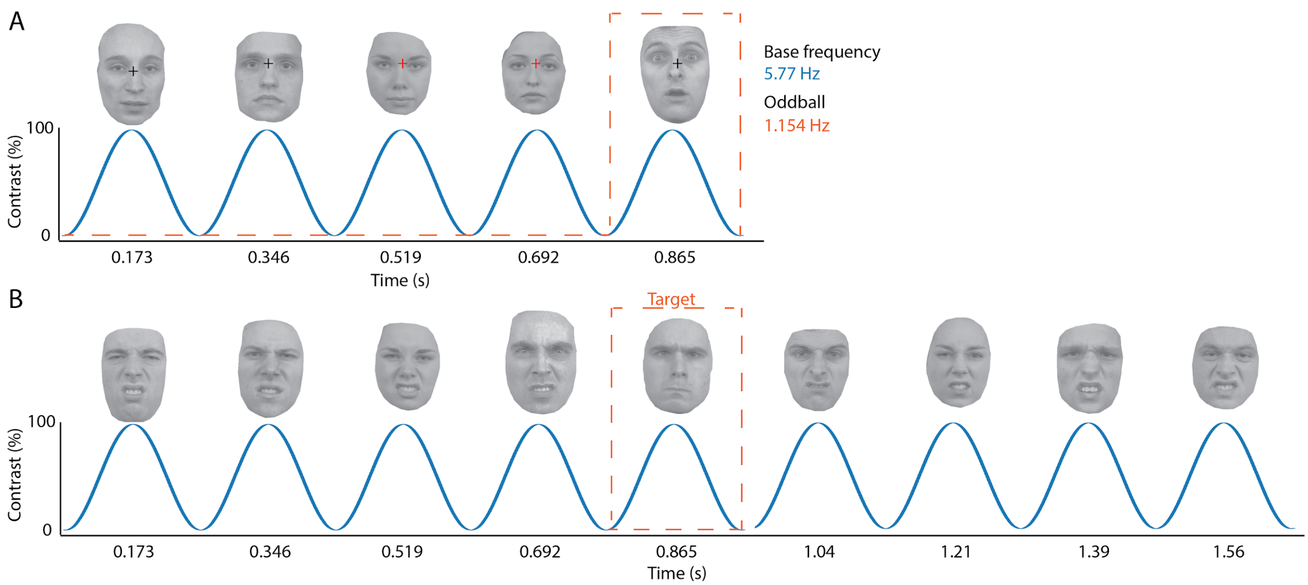

Figure 1 . A. Illustration of the implicit face categorization task. The contrast of face stimuli was sinusoidally modulated at a rate of 5.77 Hz. Each fifth frame (1.154 Hz) depicted the oddball expression. During the presentation of the faces, participants were required to pay attention to the colour of the fixation cross and to click the mouse if it turned red. Faces were presented upright or inverted for approximately 80 seconds for each of the four base-oddball emotion pairs. **B.** Illustration of the explicit categorization task. Participants were presented with a train of expressions containing an oddball expression in 50% of trials and were asked to indicate as quickly as possible if they perceived the oddball emotion.

2.2.6 Procedure. All participants took part in a single experimental session of approximately 150 minutes during which they performed several tasks as part of a larger project that will be reported elsewhere. The tasks reported here were always performed at the end of the experimental session. Questionnaire measures were administered 24 hours before the experimental session and IQ was measured before the EEG and behavioural tasks if no IQ estimate was already available for a participant. Participants performed the EEG FPVS task followed by the explicit expression discrimination behavioural task. The FPVS task was always performed first in order to measure the implicit categorization of the facial expressions before participants received the explicit instruction to discriminate the expressions in the behavioural task. Participants were instructed to remain as still as possible and to avoid eye blinks and facial movements and were allowed to take breaks between trials.

2.2.7 EEG preprocessing. All EEG preprocessing and analyses were performed using the MNE-Python toolbox (Gramfort et al., 2014) and custom functions adapted from the SSVEPY package (https://github.com/janfreyberg/ssvepy). EEG data were first high-pass filtered at 0.1 Hz and referenced to the average of all EEG electrodes. To increase the signal to noise ratio, each trial was segmented into two epochs of approximately 41 s. A Fast Fourier Transform (length = 40 s, frequency resolution = 0.025 Hz) was used to transform data in the frequency domain and the signal to noise ratio (SNR) was computed by dividing the power in each frequency bin by the mean power in 20 adjacent frequency bins (f +/- 0.25 Hz) excluding the bins immediately adjacent to the bin of interest. The SNR in each frequency bin was finally averaged across the two epochs according to the Paradigm and Orientation factors. For the sake of completeness, we also performed the same analyses on the baseline corrected amplitude (e.g. Dzheyova & Rossion, 2014), which led to a similar pattern of results. These additional analyses are reported in the supplementary materials.

3. Analyses and results

3.1 Implicit face discrimination task

Accuracy on the attentional task was above 90% for all participants (mean = 98.60%, sd = 0.02, range: 91-100%). All statistical analyses performed on EEG data employed non-parametric permutation analyses with 10000 random permutations. Analyses performed on multiple electrodes were corrected across space using threshold-free cluster enhancement (TFCE; Smith & Nichols, 2009) with a family-wise error threshold of $\alpha < 0.05$. Clusters were considered significant when at least two electrodes showed significant effects.

In order to account for any variability in the timing of the recognition of facial expressions, which may lead to responses at slightly different frequencies, the frequency bins of interest were defined as the three bins (0.075 Hz) closest to the actual Base and Oddball frequencies and their harmonics. This additional averaging step was performed given that this novel design used highly variable stimuli, which may have resulted in a 'blurring' effect across frequency bands. Averaging across frequency bins is not typically performed in FPVS studies (which thus far have included far less stimulus variability). Therefore, for maximum transparency, and to aid comparison with previous studies, we ran an analysis similar to those used previously in FPVS studies (e.g. Dzheyova & Rossion, 2014) in which a single frequency bin was analysed and analyses were restricted to *a priori*-defined sites of interest. This analysis produced the same pattern of significance as that reported here and is presented in the supplementary materials.

Based on visual inspection, the first three harmonics of the base frequency and the first five harmonics of the oddball frequency (excluding the base frequency) were considered for further analyses. For each of these bins, the effect of the Base and Oddball stimulation was tested against 1 (where signal is equal to noise) at each electrode using one-sample permutation t-tests corrected using the 'tmax' statistic (Nichols & Holmes, 2002). This analysis indicated that the SNR in all frequency bins of interest was significantly higher than 1 for at least two electrodes. All subsequent analyses were therefore performed using the sum of the Base frequency bins (centred around 5.77, 11.54, 17.31 and 23.08 Hz) and the Oddball frequency bins (centred around 1.154, 2.31, 3.46, 4.62 and 6.92 Hz) marked in Figure 2.

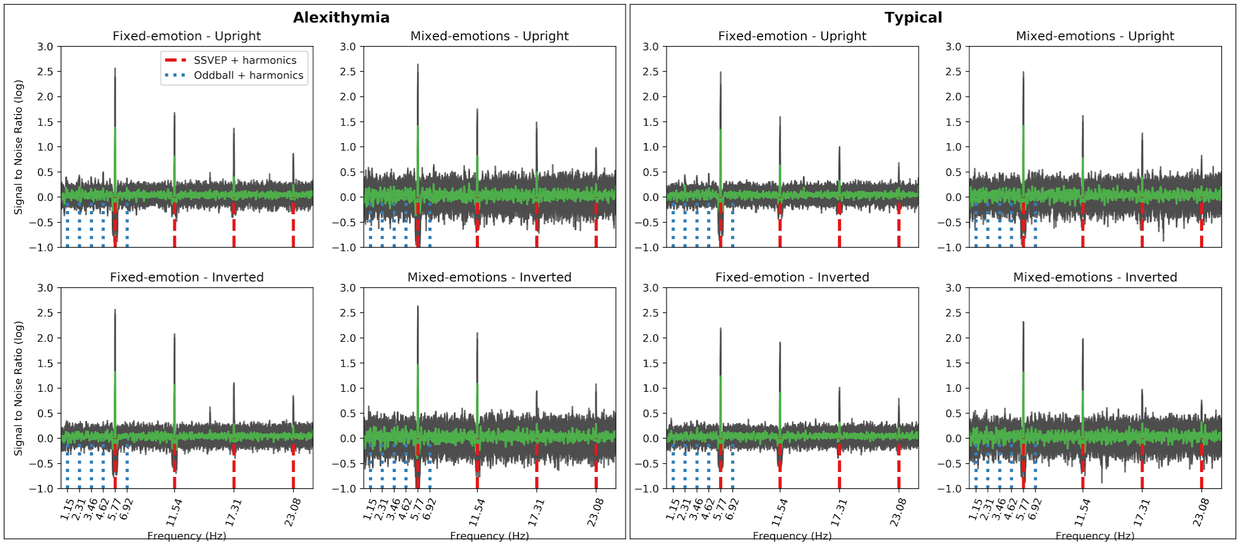

Figure 2. Signal to noise ratio for each electrode (black lines) and frequency bin for upright (top) and inverted faces (bottom) for both paradigms and groups. The green line shows the average signal to noise ratio across all electrodes and the blue and red dashed lines show the frequency bins of interest for the steady-state visual evoked potentials and the oddball frequencies, respectively. The signal to noise ratio was $\log_{(e)}$ transformed for visualisation purposes only.

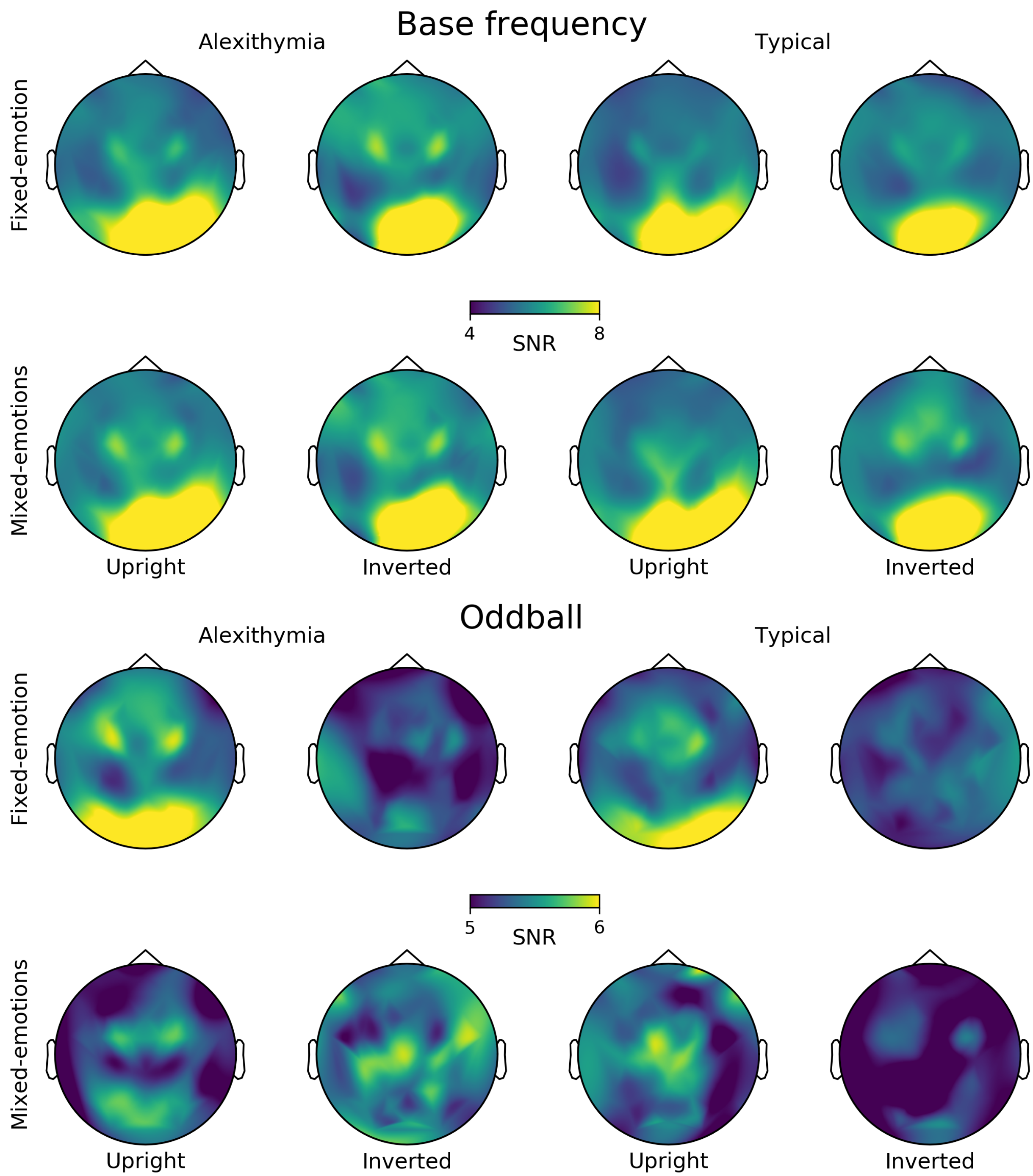

Figure 3. Summed signal to noise ratio at the base (top) and oddball (bottom) frequencies for each electrode and combination of the Group (Alexithymia, Typical) x Paradigm (Fixed-emotion, Mixed-emotions) x Orientation (Upright, Inverted) factors.

The average SNR at each electrode and in each group and condition is shown in Figure 3. To formally assess the effect of Paradigm (fixed-emotion vs mixed-emotions), Orientation (Upright vs Inverted), and Group (Typical vs Alexithymia) on SNR, we performed three-way mixed-model permutation ANOVA at each electrode separately for the base and oddball frequencies.

For the base frequencies, scalp maps indicated that SNR was higher at occipital electrodes than other electrodes across all groups and conditions. The three-way ANOVA analysis revealed a right parieto-occipital cluster showing significant main effects of Paradigm and Orientation in addition to a fronto-central cluster showing a significant Orientation effect (Figure 4). There was no significant main effect of Group or interaction at the base frequencies.

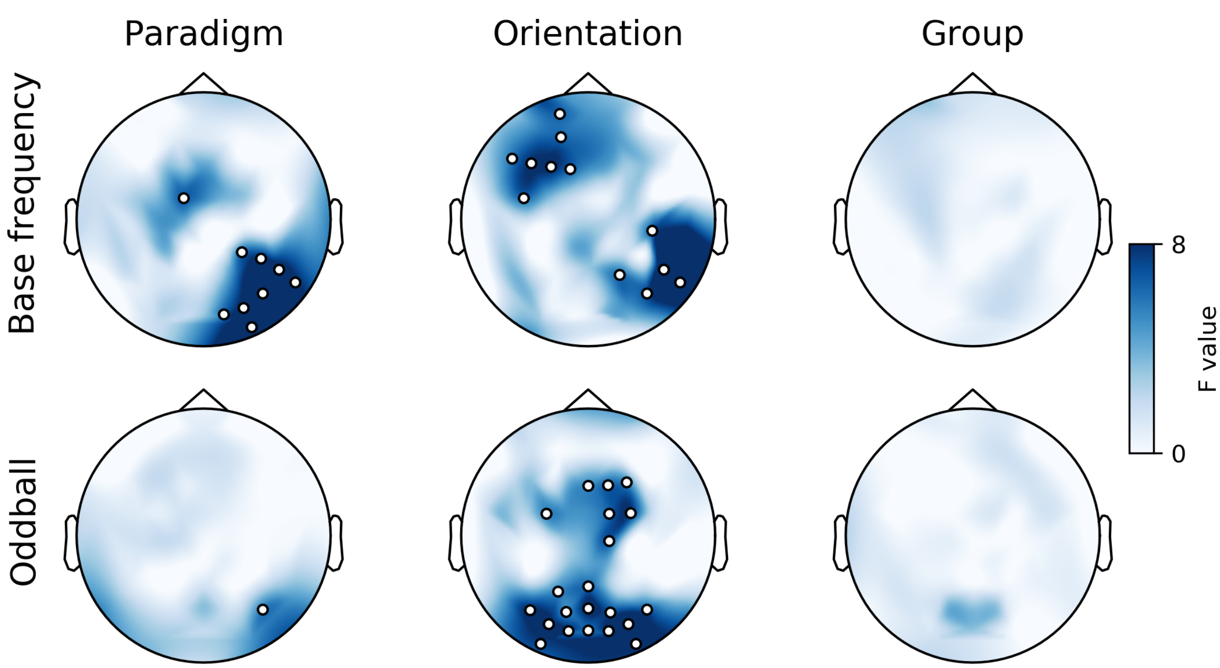

Figure 4. Main effects of Paradigm, Orientation and Group at the base (top) and oddball (bottom) frequencies. Highlighted electrodes indicate a significant effect at p<0.05 FWE corrected using TFCE.

For the oddball frequencies, in the fixed-emotion paradigm, SNR was maximal at occipital electrodes and was reduced in this cluster by the inversion of faces in both groups. In the mixed-emotions paradigm, however, the Orientation effect was more central and of a greater magnitude than in the fixed-emotion paradigm in the Typical group than in the Alexithymia group. The three-way mixed ANOVA revealed the presence of an occipital and a central cluster showing significant main effects of Orientation (Figure 4). There was no significant main effect of Group, but a significant three-way interaction was found in a central cluster of electrodes (Figure 5). In order to decompose this interaction, we carried-out a two-way Group x Orientation mixed-model ANOVA for the fixed-emotion and mixed-emotions paradigms separately. As shown in Figure 6, these analyses indicated that, in the mixed-emotions paradigm, the effect of Orientation on SNR was modulated by Group in the same central cluster, but this interaction was not present in the fixed-emotion paradigm. Bonferroni corrected pairwise permutation tests confirmed that in the mixed-emotions paradigm, inversion significantly decreased SNR relative to the upright condition in this central cluster in the Typical group (p_{perm} = 0.012, Cohen's d = 0.71; 95% CI: 0.08-1.33), but not the Alexithymia group (p_{perm} = 0.21, Cohen's d = -0.47; 95% CI: -1.12-0.18). For the sake of completeness, we performed the same pairwise comparisons in the fixed-emotion paradigm, which indicated that in this central cluster there was an inversion effect in the Alexithymia (p_{perm} = 0.02, Cohen's d = 0.65; 95% CI: -0.01-1.30) but not the Typical (p_{perm} = 1, Cohen's d = 0.19; 95% CI: -0.42-0.80) group.

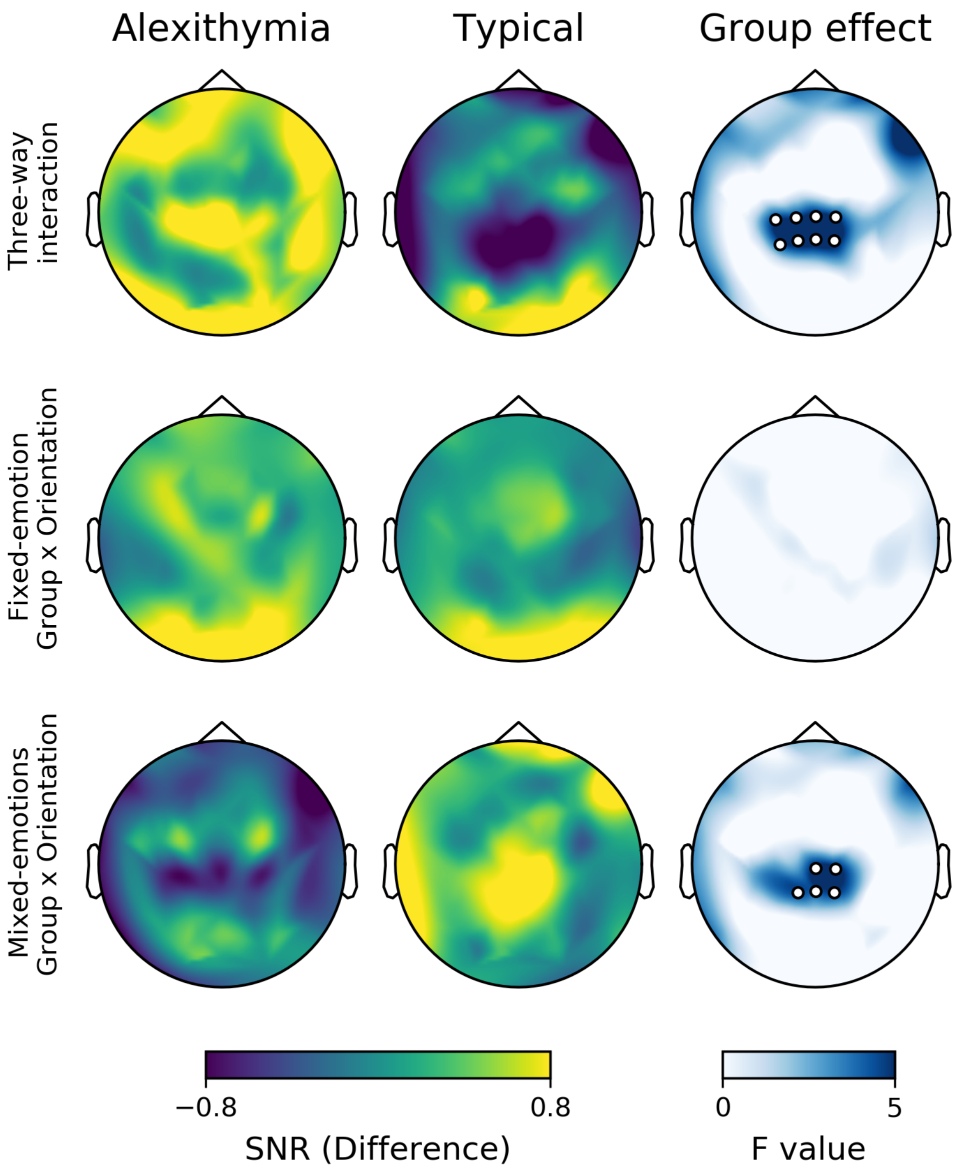

Figure 5. Descriptive scalp maps and F statistics for the three-way interaction between the Group, Paradigm and Orientation factors as well as the two-way Group x Orientation analyses performed within each paradigm at the oddball frequencies. Highlighted electrodes indicate a significant effect at p<0.05 FWE corrected using TFCE.

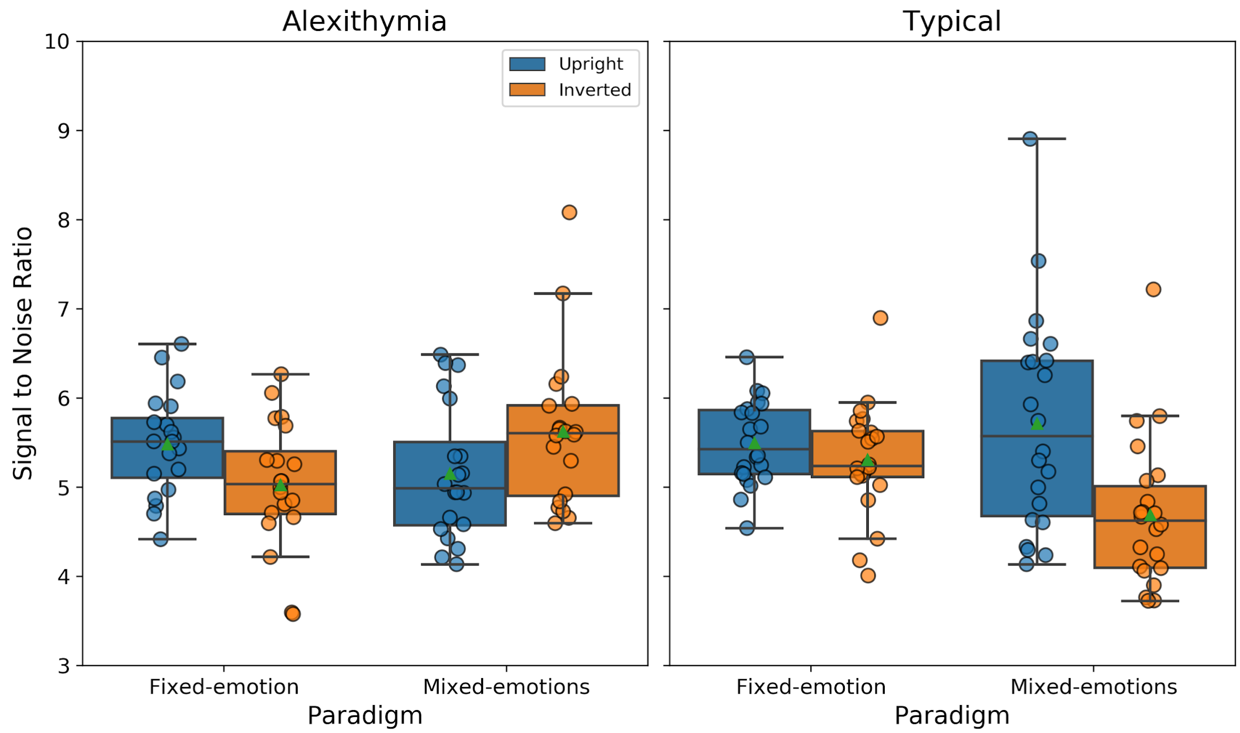

Figure 6. Summed SNR as a function of Alexithymia Group, Orientation and Trial type for the oddball frequencies for electrodes in the central cluster showing an interaction between Alexithymia and Orientation in the mixed-emotions paradigm (see Figure 4).

To guard against the possibility that any effect of paradigm at the oddball frequencies was due to the fact that the fixed-emotion paradigm had more trials than the mixed-emotions paradigm, and to test whether any particular trial type was driving the effect in the fixed-emotion paradigm, we repeated the above analyses with a three-way ANOVA in which each trial type was analysed independently (Trial type: (Fear/Neutral, Fear/Surprise, Disgust/Anger, Disgust/Random) x Orientation (Upright, Inverted) x Group (Typical, Alexithymia)). This ANOVA, and subsequent decompositions of interactions, produced a pattern of significance identical to that reported above.

3.2 Explicit face discrimination task

Participants' capacity to detect the target facial expression presented among the base expressions in the explicit discrimination task was assessed by calculating d-prime in each of the four base/target pair

Conditions (Fear in Surprise, Surprise in Fear, Anger in Disgust and Disgust in Anger). Perfect scores were corrected by subtracting and/or adding 1/(2*40 trials) to the probability of hit and/or false alarms (Macmillan & Creelman, 2004). Response time (RT) in the categorisation task was measured as the delay between the onset of the response prompt and the participant's key press. Behavioural data are shown in Figure 7.

D-prime and response time data were inspected for normality, outliers and homoscedasticity. The distributions of both variables were right-skewed and Shapiro-Wilk tests indicated significant deviations from normality for d-prime ($W = 0.98$, $p = 0.02$) and RT ($W = 0.90$, $p < 0.001$). The effects of Alexithymia and Condition on d-prime and RT were therefore assessed using non-parametric permutation two-way mixed ANOVAs with 10000 random permutations and an alpha threshold of 0.05.

The permutation ANOVA performed on the d-prime data indicated a significant main effect of Condition ($p_{perm} < 0.001$) but no significant main effect of Alexithymia ($p_{perm} = 0.66$) or interaction between the factors ($p_{perm} = 0.56$). Bonferroni corrected pairwise paired permutation t-tests indicated that the main effect of Emotion was due to a significantly higher d-prime in the Disgust/Anger trials compared to the Anger/Disgust ($p_{perm} = 0.001$, Cohen's $d = 0.90$; 95% CI: 0.45-1.36), Fear/Surprise ($p_{perm} = 0.014$, Cohen's $d = 0.52$; 95% CI: 0.08-0.97) and Surprise/Fear ($p_{perm}= 0.0012$, Cohen's $d = 0.82$; 95% CI: 0.37-1.27) conditions. The same analysis performed on the RT data indicated that there was no significant effect of Alexithymia ($p_{perm} = 0.48$), Condition ($p_{perm} = 0.13$) or significant interaction between the two factors ($p_{perm} = 0.17$).

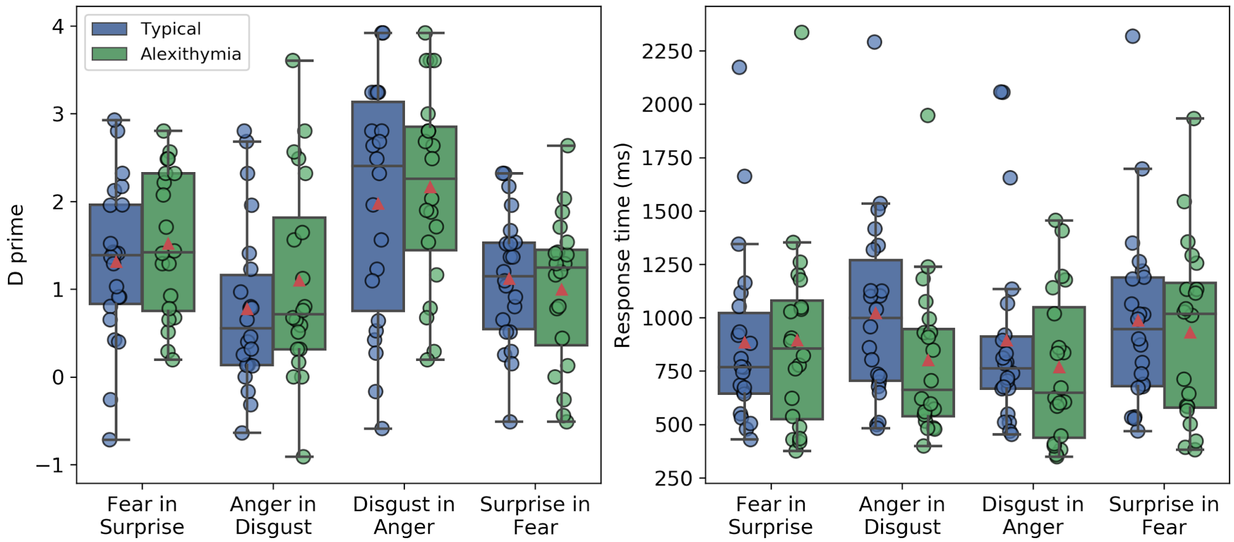

Figure 7. D-prime and response time in the Explicit face categorisation task as a function of Group (Alexithymia, Typical) and Trial type.

4. Discussion

FPVS studies are a useful method to detect neural responses relating to stimulus discrimination. If oddball stimuli (Stimulus A) are interspersed within a stream of base stimuli (Stimulus B), then any neural responses recorded at the oddball frequency index the fact that stimulus A and B have been discriminated. Where Stimulus A and B can be discriminated on one property only, the property indexed by the oddball neural response can be easily inferred. When stimulus A and B can be discriminated on a number of different levels, however, the interpretation of the oddball response is more difficult. Problematically, images of facial expressions of emotion can be discriminated on a number of levels, e.g. basic image properties (low-level visual processing), expressions, i.e. combinations of features (face-specific visual processing), and emotion (emotional-semantic processing), and therefore it is difficult to determine which psychological process is indexed by standard paradigms used to assess facial emotion discrimination using FPVS. Additionally, even if neural responses are generated at the level of full emotional-semantic recognition, it is unclear in standard FPVS paradigms whether the oddball response is generated as a result of *recognition* of the oddball stimulus, or in response to detection of a *change* from the base stream of standard stimuli.

The current study utilised two novel FPVS paradigms to address these ambiguities; a fixed-emotion paradigm and a mixed-emotions paradigm. Both paradigms introduced more visual variability into the stream of standard base images than in previous studies, thereby reducing the number of dimensions upon which oddball stimuli could be differentiated from the standard images. In the fixed-emotion paradigm, base images depicted a single emotion and oddball stimuli were of a different emotion, but successive expressions were formed by different facial identities, meaning that low-level visual properties varied at every presentation. In the mixed-emotions paradigm, each successive base image depicted a different emotional expression, formed by a different facial identity. Oddball images were comprised of different identities expressing the same emotion (disgust). Importantly, as both emotion and identity (and therefore visual characteristics of the stimuli) changed on every stimulus presentation, oddball responses could only have been generated if those responses reflect *recognition* of the oddball emotional expression, rather than simply detection of change from the base stream.

Inversion of faces led to a decrease in signal to noise ratio in the oddball frequency over central and occipital regions that is consistent with previous studies (Dzhelyova et al., 2017; Dzhelyova & Rossion, 2014). Crucially, however, in a central cluster of electrodes, this inversion effect was modulated by the type of paradigm and the level of alexithymia (i.e. a three-way Orientation x Paradigm x Alexithymia Group interaction). In this cluster, it was only in the Typical group that oddball responses were greater for upright than inverted face stimuli in the mixed-emotions paradigm.These data suggest that over central areas, oddball responses reflect recognition of the emotion or expression (rather than simply detection of change from the base stream). In this central cluster, alexithymic individuals demonstrated no difference between oddball responses to upright and inverted faces. This feature of the results further suggests that the neural oddball response observed in typical individuals in the mixed-emotions paradigm reflects expression/emotion recognition; the lack of a difference in response to upright and inverted oddball stimuli in alexithymic individuals is presumably due to their difficulties with emotion recognition.

The central distribution of the inversion effect seen in typical individuals in the mixed-emotions paradigm also supports the idea that these oddball responses reflect recognition rather than detection of change from the base stream. It is consistent with the more anterior scalp distribution of neural responses to the recognition of the identity of objects or faces (Gordon, Koenig-Robert, Tsuchiya, van Boxtel, & Hohwy, 2017; Koenig-Robert & VanRullen, 2013) compared to the posterior distribution of steady-state visual stimulation (Dzhelyova et al., 2017; Leleu et al., 2018). Central ERP responses are also associated with the explicit categorisation of facial expressions (Calvo & Beltrán, 2013; Luo, Feng, He, Wang, & Luo, 2010), suggesting that oddball responses in the mixed-emotions paradigm reflect the recognition of emotion or expression rather than the detection of change from the base stream. The lack of detectable differences in the typical group between oddball responses to upright and inverted faces in the fixed-emotion paradigm in this cluster suggests that, in the fixed-emotion paradigm, the detection of the oddball stimuli may be driven by detection of visual differences indexed at occipital sites.

The current results demonstrate that oddball responses may be observed even when low-level visual differences between stimuli are not sufficient to discriminate oddballs from base stimuli. Future FPVS studies should therefore introduce low-level visual differences into the base stream. This is particularly important as previous evidence suggests a contribution of low-level visual differences to the generation of the oddball response in standard FPVS paradigms. For example, Leleu et al. (2018) found a substantial decrease in the size of the effect of emotional intensity on the oddball response once physical differences between stimulus images were taken into account. Although the effect of emotional intensity remained significant, the use of identical base stimuli appears to have allowed for a substantial contribution of low-level visual processing. Similarly, in Dzhelyova and colleagues’ (2017) study, emotion had a significant effect on oddball responses even in the inverted condition, suggesting that physical differences between facial expressions, discriminable at a low visual level even when inverted, contributed to neural responses.

More importantly, these results also suggest that detection of change when oddball stimuli are presented may have contributed to the neural oddball responses observed in previous studies using standard FPVS paradigms. Even in the fixed-emotion paradigm, where facial identity was varied across base and oddball images, it is still the case that oddball images are more physically different to the base images (e.g. anger compared to disgust) than each individual base image is to other base images (e.g. anger A compared to anger B and anger C). This means that participants could use detection of change (at any of the processing levels) to discriminate oddballs from the base stream, in both upright and inverted conditions. Only the mixed-emotions paradigm in the current experiment was able to index recognition of the oddball expression or emotion. It is clear then, that future FPVS oddball studies of facial emotion processing should aim to maximise stimulus variability at every stimulus cycle. Indeed, the current data suggest that oddball responses can be reliably observed when stimulus variability is high, and that this methodology should be utilised in all subsequent studies. While FPVS studies do not enable one to distinguish between recognition of expression categories (face-specific visual level) and recognition of specific emotions (emotional-semantic level), this improved paradigm does allow one to infer recognition rather than simple detection of change from the base stream. However, this increased stimulus variability is bound to lead to increased variability in the frequency response, which was dealt with crudely here by using a wider frequency window to quantify the frequency responses. The methodological implications and theoretical significance of this variation should be assessed in future studies.

The inclusion of a group of individuals with alexithymia in this study allowed the mixed-emotions paradigm to be tested in those with poor emotion recognition. Although individuals with alexithymia experience well-documented facial expression recognition difficulties (Grynberg et al., 2012), they are able to detect physical differences between facial expressions when low-level perceptual discrimination of stimuli is sufficient (Cook et al., 2013). Intact perceptual processing of faces in alexithymic individuals was supported by the fact that alexithymia did not interact with the inversion effect observed at the occipital electrodes, suggesting that both alexithymic and typical participants were able to detect changes in expressions using visual cues. This intact capacity to detect physical differences between facial expressions is also supported in the current study by the fact that individuals with alexithymia performed typically on the explicit emotion discrimination task which utilised the fixed-emotion paradigm only. As might be expected from their typical (neural) discrimination on the implicit mixed image paradigm, alexithymic individuals were able to discriminate a target emotion from a stream of base emotions when explicitly instructed to do so. Crucially, however, alexithymic participants showed no difference between neural oddball responses in the upright and inverted condition in the central cluster for the mixed-emotions paradigm. This suggests that those who struggle to process the emotional content of faces used detection of visual changes, rather than expression or emotion recognition, in generating oddball responses. Interestingly, the three-way interaction observed in the central cluster was caused, at least in part, by a relatively higher response to inverted faces in the Alexithymia group than the control group in the mixed-emotions paradigm. Although speculative, it is possible that the emotion recognition difficulties of those with alexithymia lead to more reliance on individual features in daily life, leading to greater expertise in differentiating faces based on these local features, and therefore greater oddball responses in the inverted condition (when feature-based processing is used). It should be noted, however, that this difference did not reach statistical significance and should therefore be interpreted with caution and further investigated in future studies.

These findings are likely to generalise to facial identity discrimination. Instead of utilizing one facial identity image as a base image, future work should investigate whether the neural oddball response can still be observed when a) base stimuli are different images of the same individual (e.g. shown from different viewpoints, or expressing different emotions; an identity version of the fixed-emotion paradigm) and b) base images depict a number of different individuals, with a single individual (again shown in different images) as the oddball (a mixed-identities paradigm). If facial identity is truly being recognised, oddball responses should still be observed when one individual is presented as an oddball among multiple other individuals. Determining whether this is the case for facial identity as well as emotion will strengthen the conclusions that can be drawn from FPVS oddball studies. Notably, despite the increased memory demands in the mixed-emotions paradigm relative to previous FPVS paradigms (as the detection of repetition of emotion across 5 stimuli was arguably required to elicit an oddball response, rather than simply a detection of change from the base stream), the observation of oddball responses suggests that this increase was not so great that recognition was impaired.

Beyond their methodological implications, these findings add to the currently limited literature on the precise nature of the impairment that those with alexithymia experience in emotion recognition. While emotion recognition difficulties have been widely documented, it has been unclear thus far whether alexithymia represents a difficulty verbally labelling emotional expressions, or recognizing emotional expressions beyond linguistic labels. As language likely plays a role in categorising facial expressions (Barrett, 2006; Barrett, Lindquist, & Gendron, 2007; Izard, 2009; Lindquist, Gendron, Oosterwijk, & Barrett, 2013), and alexithymia was originally defined in terms of difficulties verbally labelling one's own emotions, it was initially argued that those with alexithymia may simply struggle to verbally label others' facial expressions. Deficits verbally labeling facial expressions in those with alexithymia have been observed in multiple studies (Jessimer & Markham, 1997; Montebarocci, Surcinelli, Rossi, & Baldaro, 2011; Swart, Kortekaas, & Aleman, 2009), and taking verbal abilities into account appears to reduce the effect of alexithymia on emotion recognition (Montebarocci et al., 2011), but studies have also found emotion recognition impairments in non-verbal emotion matching to targets tasks (e.g. Lane et al., 1996; Prkachin et al., 2009). As these ostensibly non-verbal tasks involved explicit emotion processing, however, verbal labelling ability may have contributed to performance (see Baldo, Paulraj, Curran, & Dronkers, 2015). To our knowledge, only the study by Cook and colleagues (2013) has attempted to distinguish between detection of visual changes and emotion or expression recognition (finding that those with alexithymia were impaired at recognising emotions, but performed typically when asked to determine whether two facial expression images were the same or subtly different), but again this study involved an explicit discrimination task, meaning language abilities may still have played a role. The current study is the first to test implicit facial expression processing in alexithymia in a truly non-verbal task, and still observed an emotion recognition impairment in those with alexithymia.

In conclusion, FPVS is a useful and efficient technique for determining implicit emotion recognition ability across multiple populations, and the present findings build upon previous work by providing a methodological technique that allows one to infer recognition, rather than simple detection of change. Future studies of facial discrimination using FPVS with oddballs should select stimuli such that a different image is used at each cycle, and emotion paradigms should also vary the emotion in the base stream, keeping only the emotion at the oddball frequency constant (though again shown in different images). A necessary future direction for FPVS research should be to determine whether the mixed-emotions paradigm can be generalised to facial identity recognition. It is likely that typical participants will show a neural oddball response to Identity A (across multiple different images) when it as shown periodically within a stream of base stimuli depicting multiple other facial identities. If this is the case, future FPVS oddball studies should use this approach, in order to conclude that facial identity has been recognised.

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Conflicts of interest

The authors declare no conflicts of interest.

Pre-registration

No part of the current study procedures or analysis was pre-registered prior to the research being conducted.

Stimuli and code

Experimental stimuli and analysis code may be obtained by contacting the corresponding author.

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Figures and tables captions

Figure 1 . A. Illustration of the implicit face categorization task. The contrast of face stimuli was sinusoidally modulated at a rate of 5.77 Hz. Each fifth frame (1.154 Hz) depicted the oddball expression. During the presentation of the faces, participants were requested to pay attention to the color of the fixation cross and to click the mouse if it turned red. Faces were presented upright or inverted for approximately 80 seconds for each of the four base-oddball emotion pairs. **B.** Illustration of the explicit categorization task. Participants were presented with a train of expressions containing an oddball expression in 50% of trials and were asked to indicate as quickly as possible if they perceived the oddball emotion.

Figure 2. Signal to noise ratio for each electrode (black lines) and frequency bin for upright (top) and inverted faces (bottom) for both paradigms and groups. The green line shows the average signal to noise ratio across all electrodes and the blue and red dashed lines show the frequency bins of interest for the base frequency and the oddball frequencies, respectively. The signal to noise ratio was $\log_{(e)}$ transformed for visualisation purposes only.

Figure 3. Summed signal to noise ratio at the base (top) and oddball (bottom) frequencies for each electrode and condition of the Group (Alexithymia, Typical) x Paradigm (Fixed-emotion, Mixed-emotions) x Orientation (Upright, Inverted) design.

Figure 4. Main effects of Paradigm, Orientation and Group at the base (top) and oddball (bottom) frequencies. Highlighted electrodes indicate a significant effect at $p<0.05$ FWE corrected using TFCE.

Figure 5. Descriptive scalp maps and F statistics for the three-way interaction between the Group, Paradigm and Orientation factors as well as the two-way Group x Orientation analyses performed within each paradigm at the oddball frequencies. Highlighted electrodes indicate a significant effect at $p<0.05$ FWE corrected using TFCE.

Figure 6. Summed SNR as a function of Alexithymia Group, Orientation and Trial type for the oddball frequencies for electrodes in the central cluster showing an interaction between Alexithymia and Orientation in the mixed-emotions paradigm (see Figure 5).

Figure 7. D-prime and response time in the Explicit face categorisation task as a function of Alexithymia group and Emotion.

Table 1. Demographic characteristics and Alexithymia scores for each group. For quantitative variables, mean and standard deviation are shown.

Supplementary Materials

Coll, Murphy, Catmur, Bird and Brewer (2019), *"The importance of stimulus variability when studying face processing using Fast Periodic Visual Stimulation: A novel 'Mixed-Emotions' paradigm"*

S1. Additional analyses and results

S1.1 Baseline corrected amplitude of EEG responses

In line with previous fast periodic visual stimulation studies using multiple quantifications of the neural response at the frequencies of interest (e.g. Collins, Robinson & Behrmann, 2018; Dzhelyova & Rossion, 2014), the same analyses performed on the signal to noise ratio at the frequencies of interest were performed here on the baseline corrected amplitude. The baseline corrected amplitude was calculated by subtracting the average amplitude of the 20 surrounding bins (excluding the immediately adjacent bin and the bins themselves) from the amplitude at each frequency of interest. As shown in the figures below, similar results were observed using this alternative dependent variable. Namely, the three-way interaction between Group, Inversion and Paradigm was also present over central sites.

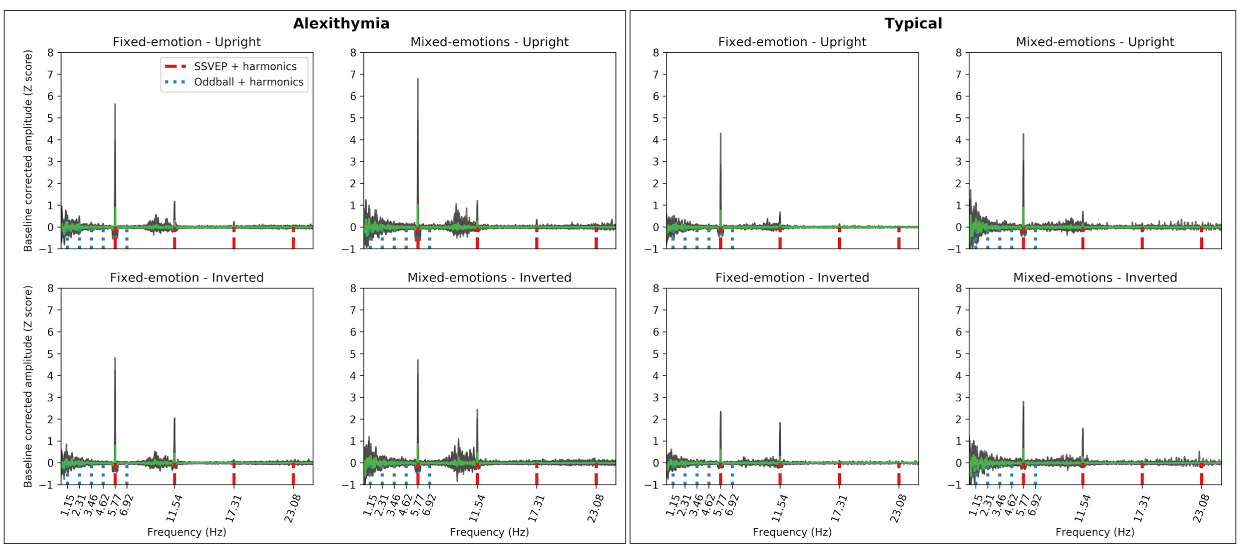

Figure S1. Baseline corrected amplitude for each electrode (black lines) and frequency bin for upright (top) and inverted faces (bottom) for both paradigms and groups. The green line shows the average signal to noise ratio across all electrodes and the blue and red dashed lines show the frequency bins of interest for the steady-state visual evoked potentials and the oddball frequencies, respectively. The baseline corrected amplitude was z scored across conditions within each electrode and participant for visualisation purposes only.

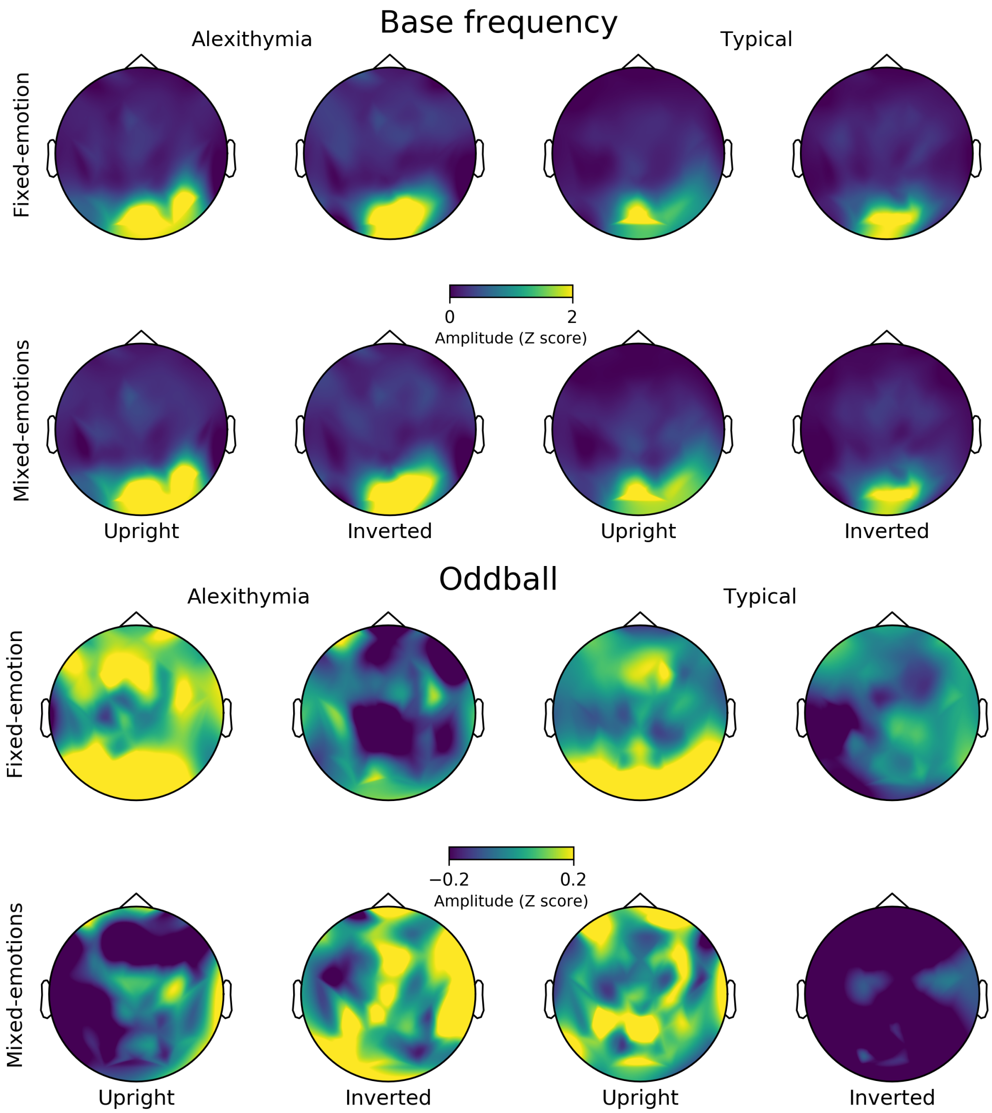

Figure S2. Summed baseline corrected amplitude at the base (top) and oddball (bottom) frequencies for each electrode and condition of the Group (Alexithymia, Typical) x Paradigm (Fixed-emotion, Mixed-emotions) x Orientation (Upright, Inverted) design. The baseline corrected amplitude was z scored across conditions within each electrode and participant for visualisation purposes only.

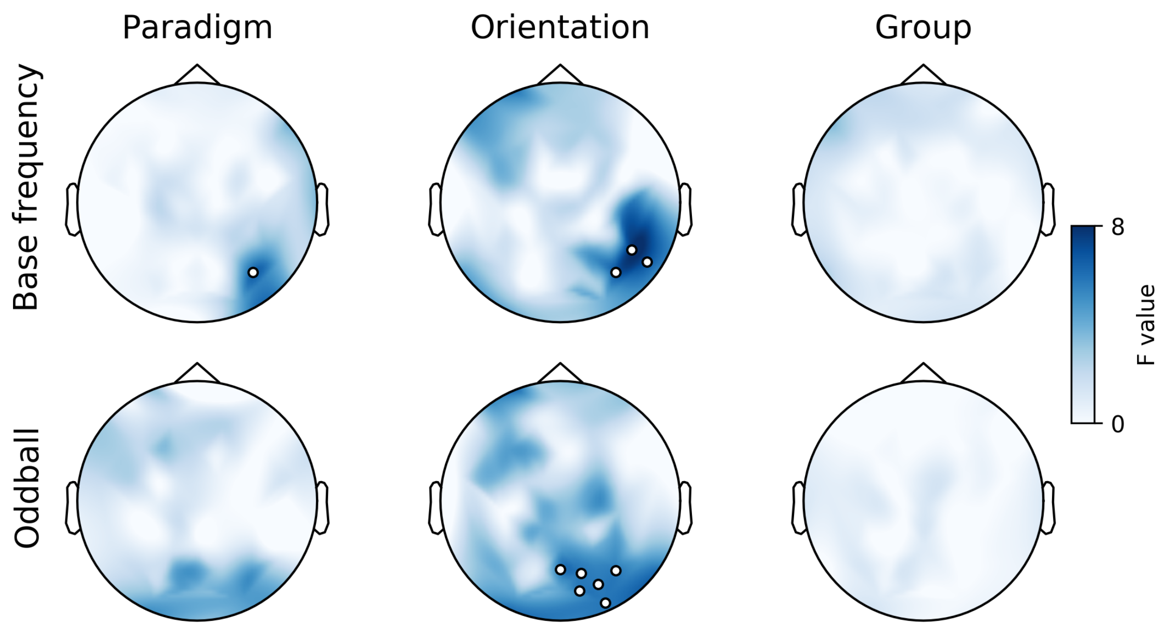

Figure S3. Main effects of Paradigm, Orientation and Group at the base (top) and oddball (bottom) frequencies. Highlighted electrodes indicate a significant effect at p<0.05 FWE corrected using TFCE.

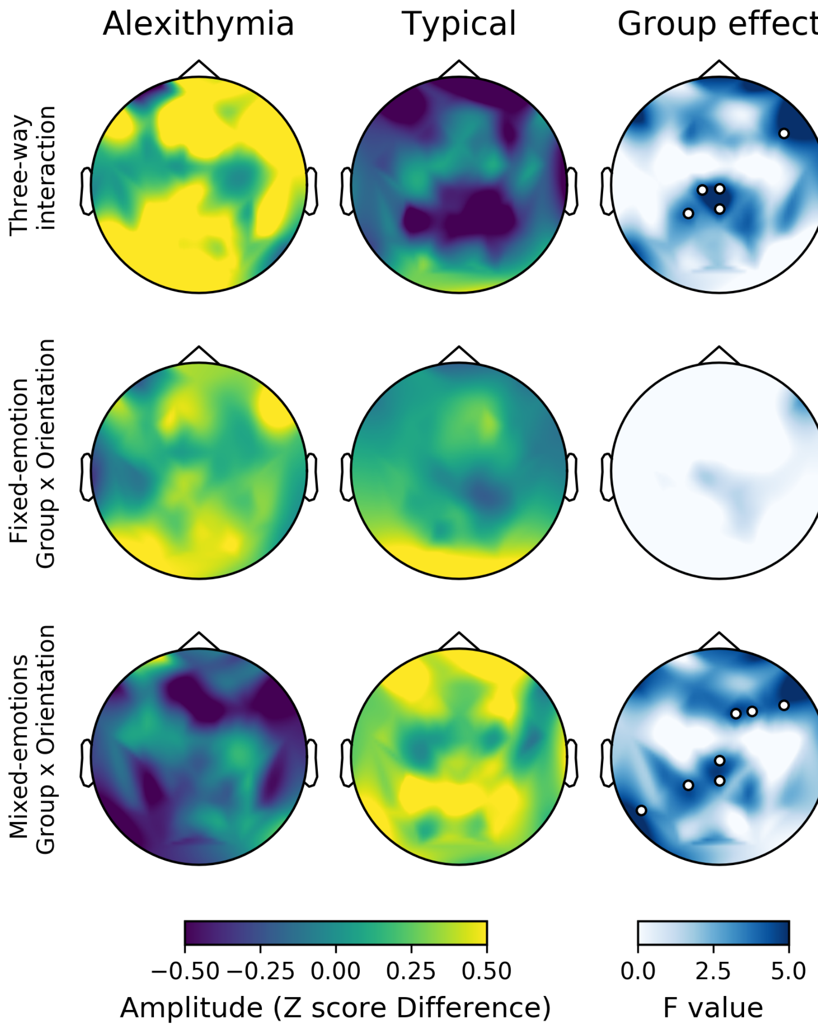

Figure S4. Descriptive scalp maps and F statistics for the three-way interaction between the Group, Paradigm and Orientation factors as well as the two-way Group x Orientation analyses performed within each paradigm at the oddball frequencies. Highlighted electrodes indicate a significant effect at p<0.05 FWE corrected using TFCE.

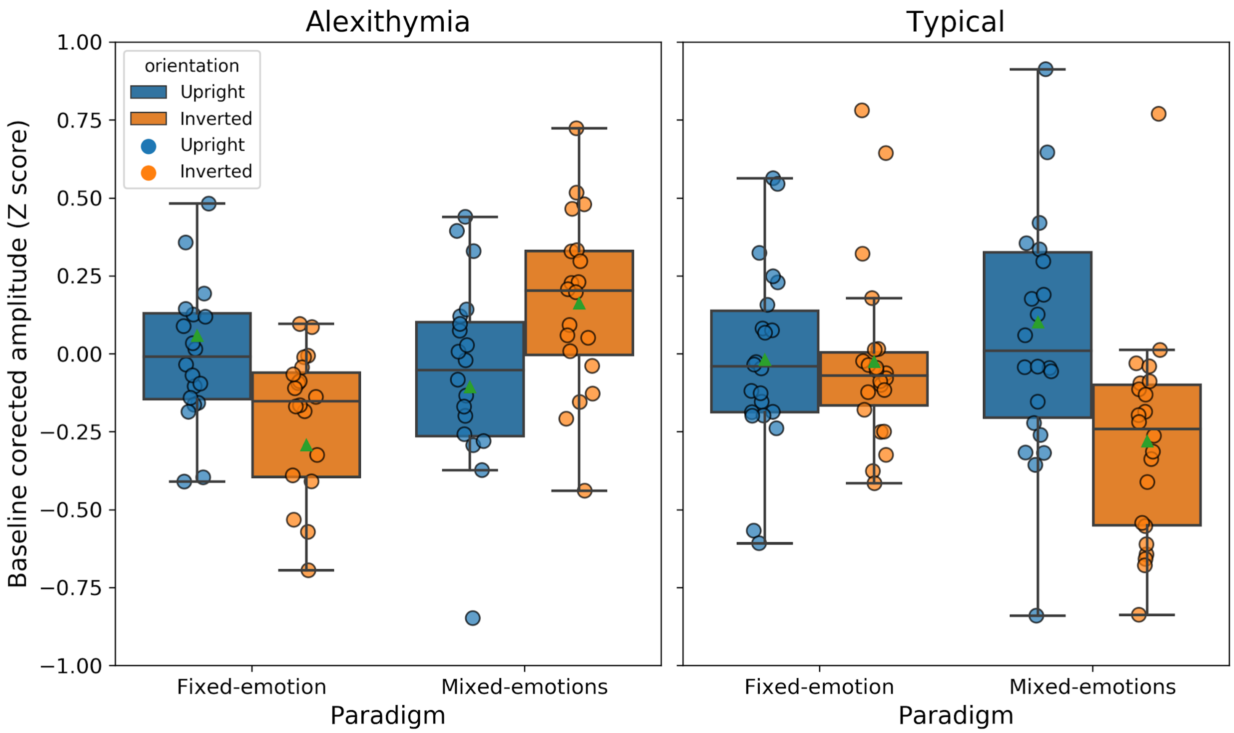

Figure S5. Summed baseline corrected amplitude as a function of Alexithymia Group, Orientation and Trial type for the oddball frequencies for electrodes in the central cluster showing an interaction between Alexithymia and Orientation in the mixed-emotions paradigm (see Figure 4).

S1.2. Analyses in ROIs without averaging across frequency bins

In the main analyses, we averaged the signal to noise ratio across the three bins closest to the frequencies of interest to account for the variability in the frequency response. Since this averaging step is not traditionally performed in FPVS studies, we report an analysis similar to those used previously in FPVS studies (e.g. Dzheyova & Rossion, 2014) in which a single frequency band was analysed and analyses were restricted to a priori-defined regions of interest (Figure S7). This analysis produced the same pattern of significance as that reported previously and is presented in the supplementary materials.

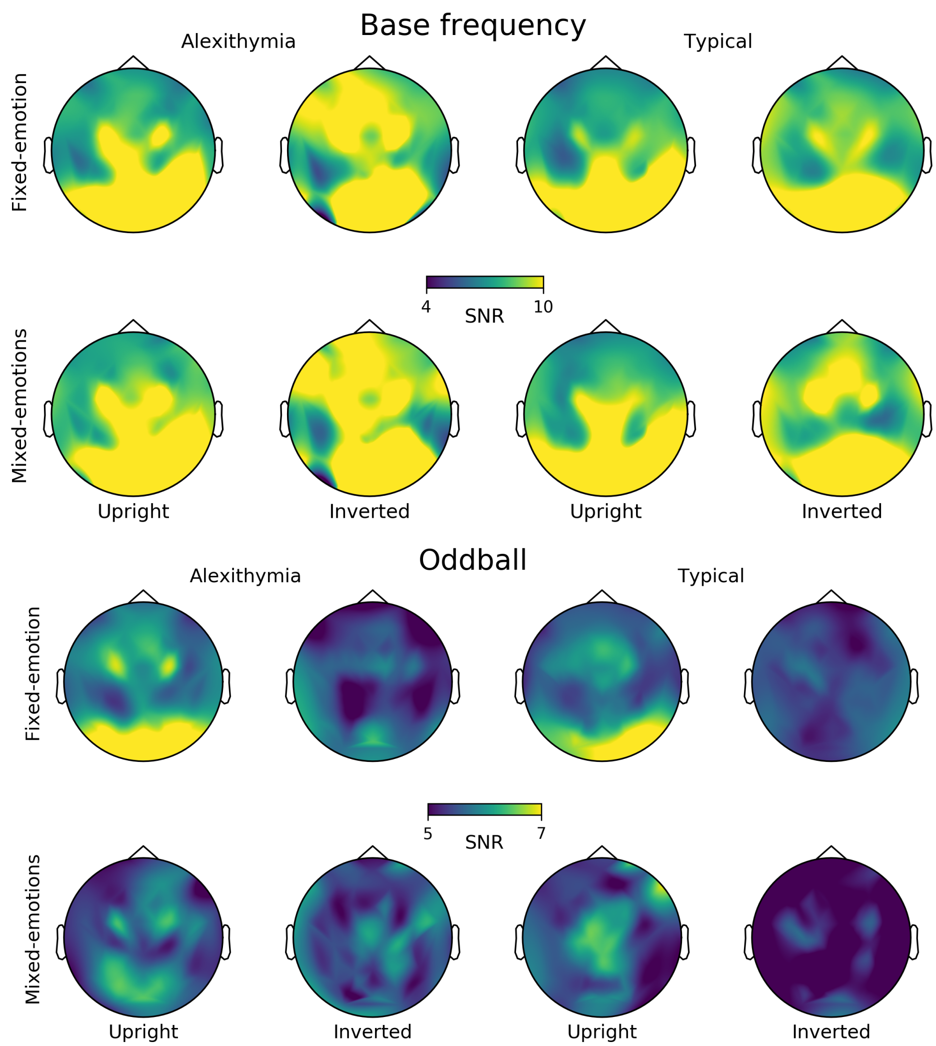

Figure S6. Summed signal to noise ratio at the base (top) and oddball (bottom) frequencies for each electrode and condition of the Group (Alexithymia, Typical) x Paradigm (Fixed-emotion, Mixed-emotions) x Orientation (Upright, Inverted) design.

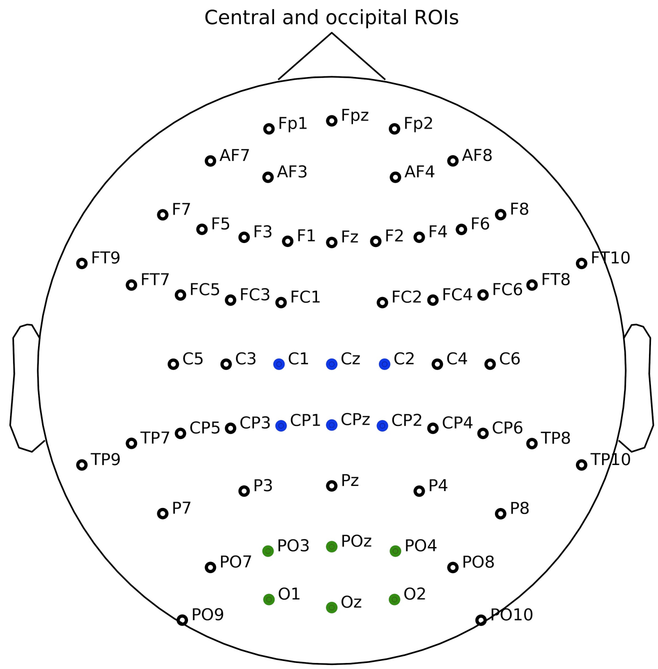

Figure S7. Scalp map showing the channels included in the ROI analyses. Two ROIs of six channels were selected. An occipital ROI (green, O1, Oz, O2, PO3, POz, PO4) and a central ROI (blue, C1, Cz, C2, CP1, CPz, CP2).

We analysed the effect Paradigm (fixed-emotion vs mixed-emotions), Orientation (Upright vs Inverted), Group (Typical vs Alexithymia), and ROI (Occipital, Central) on SNR using a four way mixed-model ANOVA.

This analysis led to a similar pattern of results as the analyses presented in the main manuscript (Figure S8). Indeed, a four way interaction [$F(1, 40) = 6.37$, $p = 0.02$, $\eta^2 = 0.01$] revealed that the a Group*Orientation*Paradigm interaction was present at the central ROI [$F(1, 40) = 4.59$, $p = 0.04$, $\eta^2 = 0.02$] but not the occipital ROI [$F(1, 40) < 1$]. As in the main text, Bonferroni corrected pairwise comparisons confirmed that for the mixed-emotions paradigm, there was a significant main effect of inversion in the Typical group ($p = 0.047$) but not the Alexithymia group ($p = 1$).

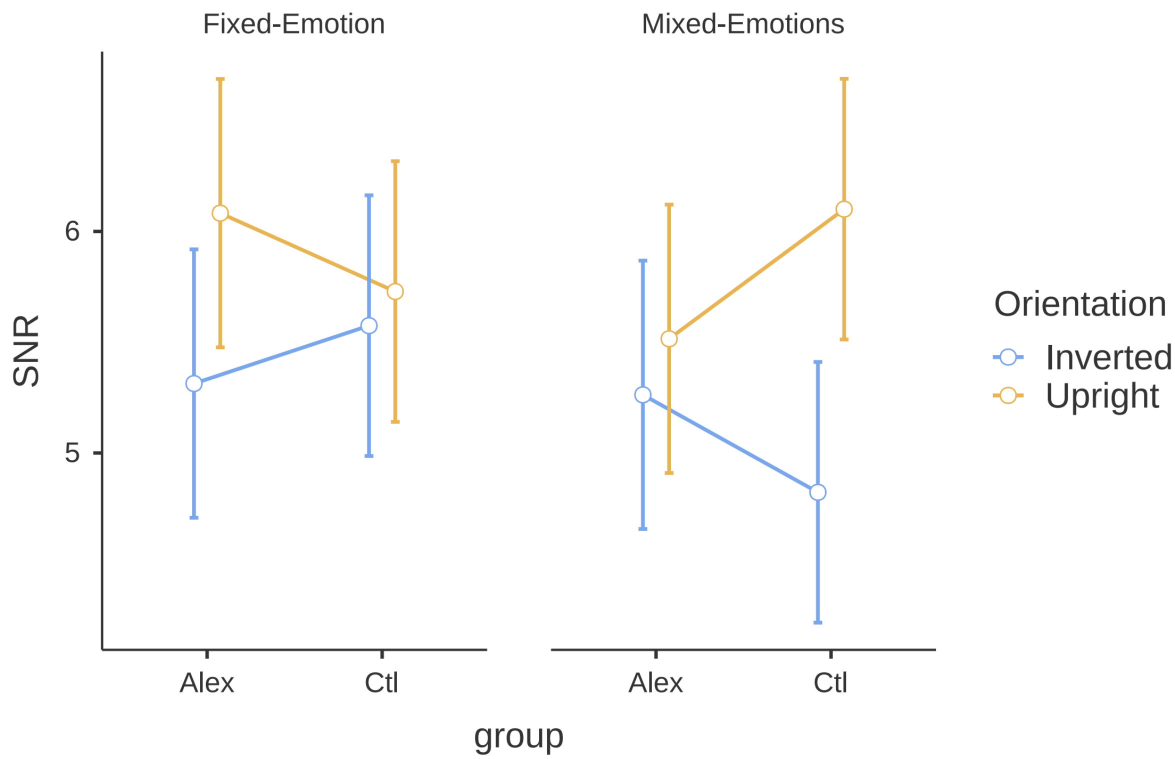

Figure S8. Summed SNR as a function of Alexithymia Group, Orientation and Paradigm for the oddball frequencies for electrodes in the central region of interest. Error bars show standard error.

Supplementary references

Collins, E., Robinson, A. K., & Behrmann, M. (2018). Distinct neural processes for the perception of familiar versus unfamiliar faces along the visual hierarchy revealed by EEG. *NeuroImage*, *181*, 120-131.

Dzhelyova, M., & Rossion, B. (2014). The effect of parametric stimulus size variation on individual face discrimination indexed by fast periodic visual stimulation. *BMC neuroscience*, *15*(1), 87.